PESTS NOT KNOWN TO OCCUR IN THE UNITED STATES OR OF LIMITED DISTRIBUTION, NO. 28: CITRUS BLACK SPOT

Prepared by USDA, APHIS, PPQ, Biological Assessment Support Staff, Federal Building Room 626, Hyattsville, MD 20782

Class: Ascomycetes

Order: Family: Sphaeriales: Sphaeriaceae

Pest: CITRUS BLACK SPOT (CBS)
Teleomorph *Guignardia citricarpa* Kiely 1948

Anamorph *Phoma citricarpa* McAlpine 1899 or *Phyllostictinia citricarpa* (McAlpine) Petrak 1953

(Teleomorph = sexual stage, anamorph = asexual stage)

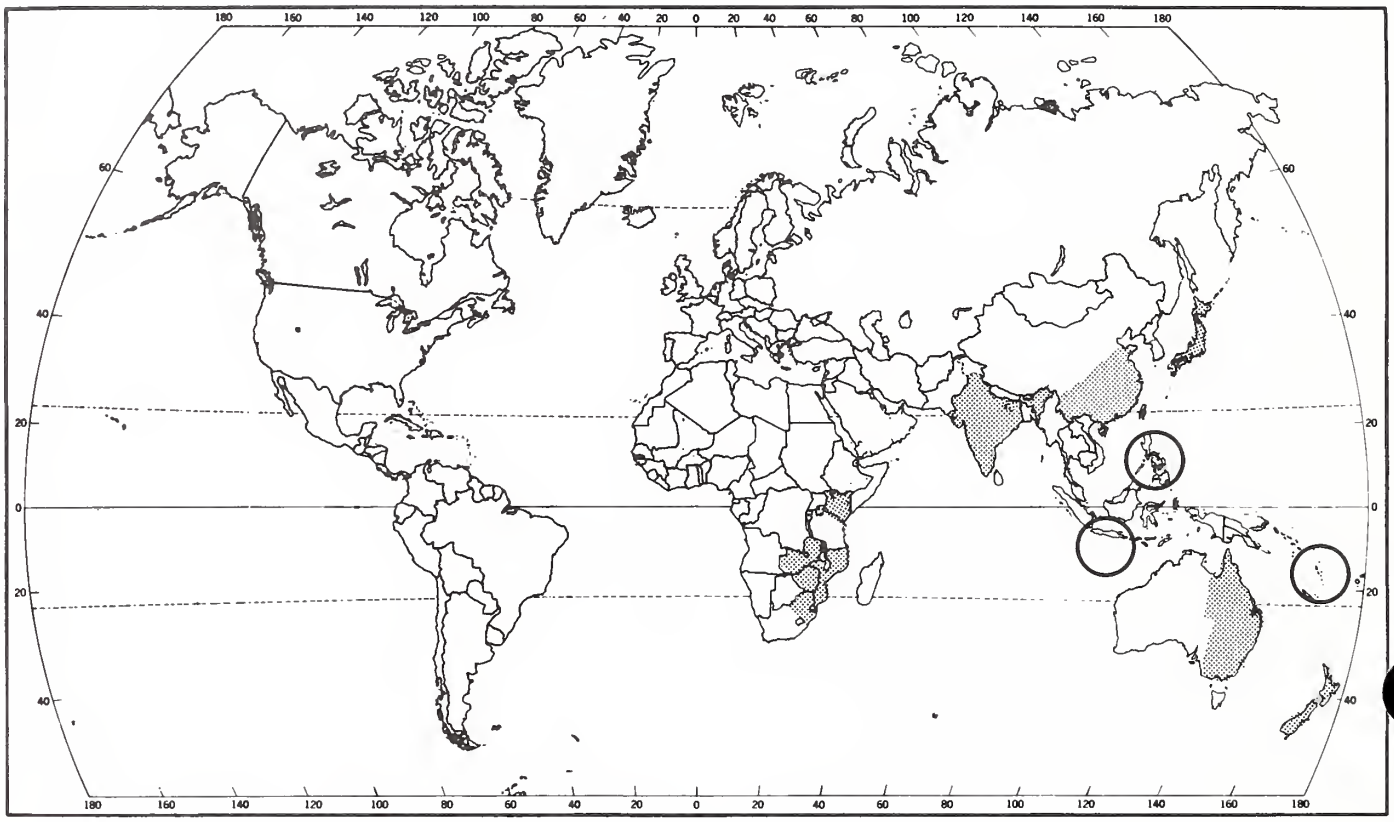
Economic Importance: CBS is a serious disease of the rind affecting all commercial varieties (except sour orange) of mature citrus fruits in the orchard and during storage (Hall 1973).

Hosts: All commercial *Citrus* spp. are susceptible except *C. aurantium* (sour orange). *Citrus limon* (lemon) is particularly susceptible. Losses may be heavy in the Valencia and Navel varieties of *C. sinensis* (sweet orange). *Fortunella* spp. (kumquats) are also susceptible (Kotze 1981, personal communication).

General Distribution: Australia (New South Wales, Queensland), China (southeast), Hong Kong, India, Indonesia (Java), Japan, Kenya, Mozambique, New Hebrides, New Zealand, Philippines, South Africa (Natal and Transvaal, not in Capetown Province), Swaziland, Taiwan, Zambia, and Zimbabwe. The disease has been intercepted at U.S. ports from countries from which there are no official reports of the disease.

There is a nonpathogenic closely related fungus (*Guignardia* sp.) which has been reported as *G. citricarpa* on hosts other than citrus at many other locations. Doubtful reports that may be due to this nonpathogenic fungus (see discussion below) have been omitted from this distribution (Kotze 1981, Commonwealth Mycological Institute 1969).

The disease was first known in Australia about 1895. CBS is confined to the coastal areas of high rainfall and principally affects the Valencia variety since this matures during warm weather, when conditions favor the disease (Hall 1973).



Guignardia citricarpa map prepared by USDA, APHIS, PPQ, Biological Assessment Support Staff

Characters

The fungus, Phoma citricarpa, was first described by McAlpine (1899) in Australia where it was constantly associated with CBS lesions. In China, this fungus was isolated by Lee (1920) in pure culture, and inoculation experiments related the fungus to the CBS lesions.

Secondary fungi often outgrow the fungus so that it is not always easily isolated. The fungus exhibits a restricted growth on beef agar + 1 (Fuller's scale), glucose agar + 1, and potato plugs. The early stages of mycelial growth are grayish, but the colony soon becomes completely black from the formation of pycnidia; colonies small and restricted. Pycnidia appear 6-7 days after plating and are abundant, black, spherical, and with pores indistinct.

As seen under the microscope, hyphae in their early stages of growth are hyaline and distinctly septate, but with age the hyphae become dark olive brown, closely septate, twisted, and swollen. Spores born apically on slender, hyaline conidiophores are hyaline, single celled, smooth, and thin walled, ovate, sometimes becoming almost pyriform, noticeably granular; from cultures they vary in size from 9.25-12.25 by 5.5-8.1 microns. Spores not formed profusely. Diagnostic features: Black, carbonaceous, restructed growth in culture, thin-walled, granular and somewhat individualistic spores. The best determination is pathogenicity on sweet oranges which may be visible in 15-20 days (Fawcett 1936).

Due to much confusion about the epidemiology (especially the role played by other hosts) and distribution of G. citricarpa, McOnie (1964) conducted a study of Guignardia in South Africa. He discovered that there are two species of Guignardia on citrus. G. citricarpa Kiely causes black spot of citrus, and host range appears to restrict it to citrus. The other is unnamed and is referred to as Guignardia sp. The latter does not cause black spot although it is found latently in citrus, and in the leaves of many other plants. In pure culture growth of Guignardia sp. differs from that of the black spot fungus. Perithecia are readily produced by the Guignardia sp., but G. citricarpa rarely produces them in artificial culture. Guignardia sp. seldom produces pycnidia and spermagonia, whereas G. citricarpa produces them freely in culture and on dead leaves.

The fungus, Guignardia sp., is very widely distributed, and fruits from Brazil, Honduras, and Trinidad were more heavily infected than any South African fruit tested. Citrus from Argentina, Brazil, British Honduras, Florida, Honduras, Israel, Jamaica, Sicily, Spain, Trinidad, and Uruguay proved to have infection of Guignardia sp. (Kotze 1981).

Characteristic Damage

CBS is primarily a fruit disease and is found only occasionally on the leaves and twigs. First symptoms to appear are hard spots or shot-hole lesions, small reddish-brown spots on the surface of the fruit, sometimes even before the fruit turns color. Lesions on green fruit have a yellow halo. Later these spots turn darker, sometimes becoming entirely black. The spots vary in size and may be 1-2 mm in diameter, but the area may enlarge to 8-10 mm or even 15 mm which may coalesce to affect most of

the rind. As the spots mature, a reddish-brown raised margin forms around the outer edges of the spot, while the center sometimes becomes depressed and assumes a light tan or brownish color. Later there is a further sinking of the spot and the edges become darker.

Pycnidia are sometimes present in the depressed, lighter colored area. They are black and a mere fraction of a millimeter in diameter. The lesion not only occurs on the surface but extends into the skin tissue from 1 to as much as 2 mm. In this internal tissue the lesion is usually light colored, similar to the reddish brown of a young lesion. The lesion has never been observed to extend into the flesh of the fruit, although secondary rots, emanating from a black spot lesion as the original point of infection, do sometime progress into the flesh.

After the fruit turns color, freckle spots appear and may occur in more than one wave of infection on a fruit. Individual spots may coalesce to form a large lesion. A tearstain similar to melanose may be the result. In storage this lesion may develop into virulent spot. Virulent spot develops mostly on mature fruit late in the season and can cause much loss even before picking.

CBS lesions on leaves are brown circular areas of dead tissue about 2 mm in diameter. The fruiting bodies of the fungus appear in the centers of these areas. Leaf spots can develop at any time of the year, and are more common on lemon than on orange foliage (Kiely 1969).

The disease may not be abundant on fruit in the orchard, but then develops readily while it is in storage or in transit to markets. Fruits have been shipped entirely unblemished which, on unpacking 1 month later, were found severely affected by this black spot. The injury to the crop is much the same as that of other rind diseases. The blemish lessens the fruit's market value.

Detection Notes

CBS may be confused with septoria spot (Septoria citri), which is similar in appearance but differs in having lesions with a persistent reddish-purple tinge (Hall 1973). See PNKTOs No. 27 citrus canker and No. 29 sweet orange scab for other symptoms that may be confused with the symptoms incited by G. citricarpa.

(Fig. 1)

Guignardia citricarpa on Valencia orange, Nelspruit, Republic of South Africa, 9/2/68 (photo by W. H. Wheeler)

Biology

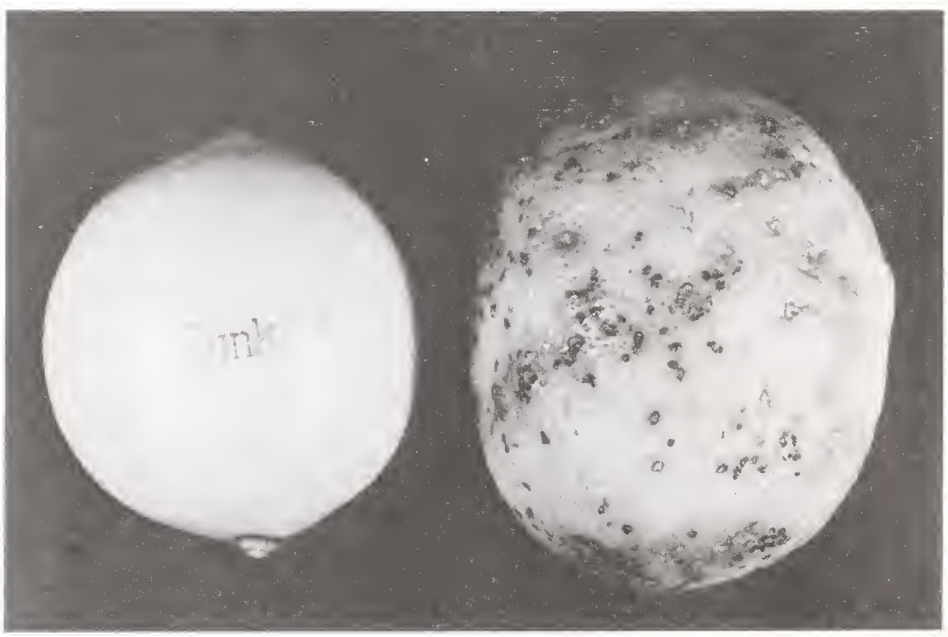
To understand the epidemiology of CBS, information is required on inoculum availability, climatic conditions required for infection, and the growth cycle of citrus, particularly fruit development as related to disease development. The relative importance of the two forms of inoculum, ascospores and pycnidiospores, was often misunderstood. Each plays a role in establishing an epidemic, but once the disease is epidemic, ascospores usually become of completely dominant importance.

Pycnidiospores may occur in fruit lesions, on dead twigs and leaves, and occasionally, on fruit stalks and in lesions on attached leaves. Pycnidiospores are produced abundantly on dead fallen citrus leaves and reach susceptible fruits only by raindrop splash. Fruit to fruit infection occurs by rain splash when mature diseased fruit still remains on the tree after petal fall.

(Fig. 2)

Guignardia citricarpa on sweet orange. Citrus and Subtropical Research Institute, Nelspruit, Republic of South Africa, 9/12/68 (photo by W. H. Wheeler)

(Fig. 3)



Guignardia citricarpa on lemon compared to healthy U.S. fruit

Ascospores produced on fallen dead leaves are the main source of inoculum once the disease has reached the epidemic stage. Investigations on CBS in Zimbabwe in 1965 could find only small numbers of ascospores in infected orchards; losses from CBS had been very slight. By 1978, an epidemic was well established and ascospores were abundant in the same areas.

G. citricarpa may be present for many years in a particular area before symptoms appear. The time the first symptoms are noticed until the disease reaches epidemic proportions may be 5-30 years, depending on the presence of lemons (which speed up the process) and climatic conditions. In western Transvaal, South Africa, symptoms were observed for over 30 years before control measures became necessary. Once epidemic, the disease remains serious unless effectively controlled. There are no examples in South Africa of CBS disappearance or decline after becoming epidemic (Kotze 1981).

Infection occurs when fruit is young. In older trees the period of susceptibility extends from blossoming to about 5 months later. In younger trees, up to 10 years old, the susceptible period is considerably shorter, about 3 months, and the disease is more easily controlled.

Infection occurs with the actual penetration of the fruit rind by the germinating fungus spore. Immediately after this penetration the fungus forms a microscopic resting body within the rind tissue; this resting body remains in a dormant condition until high air temperatures and rind maturity provide the conditions necessary for its further growth. This is known as latent infection. Fruit maturing during winter and early spring usually does not develop black spot. Although the fruit has been infected, it matures before the advent of the high temperature necessary to stimulate further fungus activity.

The factors most likely to encourage black spot development are high temperatures, low soil moisture, and aging of the rind in late spring and early summer. Hot, dry winds in spring and early summer are usually followed by a sudden development of the disease. Fruit on the sunny side of the tree is most severely spotted, though eventually the entire crop may become affected.

A number of environmental factors can influence the rate of aging of the rind, and so increase the severity of the disease. These include unbalanced soil nutrients, lack of soil moisture, and poor tree vigor. Often, fruit on trees rejuvenated by pruning does not show symptoms. However, after the fruit is picked, lesions may develop from latent infections if fruit is exposed to high temperature in storage.

In Australia, the symptoms generally do not start to develop until the weather begins to warm in August. The first signs of the disease are small rust-red depressions less than 2 mm in diameter. These are in the rind on the exposed surface of the fruit on the sunny side of the tree.

Subsequent development of spots depends upon weather conditions and age and condition of the tree. The hard spot type develops during spring weather when the temperature is not very favorable for fungus growth and are characteristic of the early or spring phase of black spot. Since there is little subsequent increase in size, and fruit-keeping quality is not affected, the disease is not serious at this stage (Kiely 1969).

In South Africa, there is a false melanose stage that precedes the hard spot stage. This is not associated with Phomopsis citri but is an early season symptom caused by G. citricarpa in that area (Kotze 1981).

Later in the spring in Australia, when the rind is fully mature and temperatures are higher, the more virulent types of spot appear. The primary rust-red pits continue to grow, producing large brown sunken lesions which may be 7-14 mm in diameter, or may involve half or more of the fruit surface. Pinpoint-size black dots appear on these lesions; the black dots are the spore-producing bodies or pycnidia of the fungus.

Development of this virulent spot type of lesion can be extraordinarily rapid--up to two-thirds of the fruit surface may be affected by the irregular growth of a single spot in 4-5 days. Affected fruit falls readily. In some cases half the crop has dropped within days of the first appearance of lesions during a spell of hot, dry weather. Later in the season, when the rind begins to regreen in late-held crops, there is little development of black spot.

Perithecia of G. citricarpa were present in dead citrus leaves throughout the year in Taiwan. The formation and development of perithecia on the fallen dead leaves of citrus were mainly influenced by temperature and rainfall.

In laboratory studies, no perithecia developed at temperatures below 7°C. At 14°C, perithecia, asci, and ascospores develop at 14, 32, and 42 days, respectively, after citrus leaves were detached and alternately wetted and dried daily. It required 10 days to form perithecia and 27 days for ascospores as temperatures ranged 21-28°C.

Moderate precipitation and an even distribution of rainy days favored the formation and development of perithecia. A large quantity of precipitation on rainy days caused the decomposition of the dead leaves before the perithecia could develop and mature, thus eradicating the latent pathogen. Prolonged dry weather inhibited the formation and development of perithecia, but did not kill the latent pathogen. Ascospore release from mature perithecia was affected apparently by the timing rather than the total amount of precipitation. At optimal temperatures (21-28°C) and with adequate precipitation, maturation of perithecia on the dead leaves took about 4-5 weeks.

When the dead leaves with mature perithecia were immersed in water, the ascospores started to release after 20-140 minutes. In most cases, ascospore discharge lasted less than 1 day. Heavy spore discharge occurred in the first day, and the number discharged declined in later days. Only a few spores were released after the 9th day.

A large quantity of ascospores could be collected in the orchard shortly after rainfall; nevertheless, a few ascospores could still be collected during fair weather, mostly at night. Dew also had some effect on the formation and development of perithecia and the discharge of ascospores (Lee and Huang 1973).

By using a Hirst spore trap for collecting ascospores during 1968-1970, Huang and Chang (1972) found the most favorable period in Taiwan for ascospore discharge occurred from the end of May to early October. Rain and high temperature were important to induce the development of perithecia and discharge of ascospore. When there were mature perithecia in an orchard, heavy dews probably were effective in inducing ascospore discharge. Summer climatic factors favored spore discharge and a large number of spore discharges occurred. Discharge decreased

to a very low level during the winter and early spring. The results of spore trapping and tissue isolation made from apparently healthy leaves indicated a coincidence of initial leaf infection with the beginning of ascospore discharge in late May.

Control

Fruit on young trees, though susceptible, is not seriously affected by black spot and control is easier on young trees. For this reason there are different programs for black spot control--one for young vigorous trees up to 10 years old, and others for older trees.

Fruit intended for export, particularly for tropical markets, should be harvested in the spring from young trees that have been protected by the full spray program. This fruit should be stored as refrigerated cargo during transit to minimize disease development. To hold fruit on trees for later harvesting, choose young or particularly vigorous trees and apply the full spray program.

Fruit should be harvested as early as practical. Pick the sunny side of the trees first because this is where the disease is most likely to start to develop. Pick all diseased fruit as soon as possible, and remove along with all fallen fruit from the orchard. Mature, diseased fruit on the trees or ground is a source of infection for the young developing fruit, at and after blossoming. Bloom is earlier than this in South Africa and, of course, is different in the Northern Hemisphere e.g. Taiwan, and a constant source of infection during the early months of development of the new season's crops.

On light soils a supplementary irrigation after the first harvest, under dry conditions, helps prevent black spot development. This should be done in addition to the spray program (Kiely 1969). For a discussion of chemical controls see Kiely 1976 and Kotze 1981.

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